

## Microbiological Comparison of Surface Soil and Unsaturated Subsurface Soil from a Semiarid High Desert

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**Thirty-two chemoheterotrophic bacteria were isolated from unsaturated subsurface soil samples obtained from ca. 70 m below land surface in a high desert in southeastern Idaho. Most isolates were gram positive (84%) and strict aerobes (79%). Acridine orange direct counts of microbes in one subsurface sample showed lower numbers than similar counts performed on surface soils from the same location (ca.  $5 \times 10^5$  versus  $2 \times 10^6$  cells per g [dry weight] of soil), but higher numbers than those from plate counts performed on the subsurface material. Another sample taken from the same depth at another location showed no evidence of colonies under identical conditions. Soil analyses indicated that subsurface sediments versus surface soils were slightly alkaline (pH 7.9 versus 7.4), had a higher water content (25.7 versus 6.3%), and had lower organic carbon concentrations (0.05 to 0.17 versus 0.25% of soil dry weight). Analyses of biologically relevant gases from the unsaturated subsurface indicated an aerobic environment. As in other unsaturated soil environments, either a high proportion of bacteria in these subsurface sediments are not viable or they are incapable of growth on conventional media under aerobic conditions. The presence and numbers of bacteria in these deep sediments may be influenced by colonization opportunities afforded by periodic percolation of surface water through fractures in overlying strata.**

The importance of unsaturated subsurface environments has to do primarily with their location with respect to groundwater supplies. Specifically, an unsaturated zone represents (i) the pathway through which contaminants may pass to reach groundwater; (ii) the site where biological, chemical, and physical factors influence or alter contaminants; and (iii) potential zones of contaminant treatment, using soil bioreactors (4, 9). Recent studies, conducted primarily in the saturated zones of humid climates, indicate the presence of diverse microbial types and activities in deep sediments (6, 15). Little is known of the microbiota that exist in deep unsaturated zones of arid climates, and yet many existing or proposed hazardous waste sites are located in or near such environments (e.g., New Mexico, Nevada, Washington, and Idaho), thus presenting the possibility of microbial interaction with waste materials. The possibility exists that infrequent saturating conditions in surface soils may allow transport of materials including bacteria from the surface to deep strata via percolation water (2, 14).

Based on recent evidence that microbes exist in deep, saturated strata and on the possibility that percolation water may transport bacteria, it was hypothesized that viable microbes exist in deep, unsaturated strata in an arid climate. To this end, the presence of microbes in deep, unsaturated sediments was determined by direct and plate counts. Bacterial isolates provided preliminary evidence of the diversity of subsurface microorganisms from this environment. Further, the pH, organic carbon, water content, and primary gaseous constituents in subsurface samples were measured to determine the suitability of this environment for microbial survival.

The sampling sites were in a local drainage basin at the Radioactive Waste Management Complex within the Idaho National Engineering Laboratory in south central Idaho. The eastern Snake River Plain is a semiarid high desert (ca. 1,500 m above sea level) with a mean annual precipitation of 20 cm (14). Approximately 9 m of surficial soils (primarily silt, sand, and clay) overlies subsurface basalts which com-

prise ca. 95% of the subsurface material (Fig. 1). Basalt flows alternate with thin sediment beds consisting of clayey silt at nominal depths of 9, 34, and 73 m. The aquifer is at a depth of 177 m.

Drilling was conducted by using an air rotary method with forced, HEPA-filtered air to remove cuttings. Drilling muds were not used. Sediment beds were cored slowly to reduce heating of the core. The surface of a fresh sediment core was shaved with a sterile spatula and then subcored with a sterilized 60-ml plastic syringe with the end removed. The subcore (ca. 40 g, wet weight) was then extruded into a sterile Whirlpak bag which was sealed. Samples were stored at 4°C, and analyses were initiated within 18 h of time of collection. Abiological analyses were conducted on core shavings.

Three samples were obtained from the subsurface: (i) well D15, September 1987, depth of 70 m; (ii) well 8801D, July 1988, depth of 70.1 m; and (iii) well 8801D, July 1988, depth of 70.4 m. Of the latter two, one (70.1 m) was obtained from the basalt-sediment interface, while the other (70.4 m) was obtained deeper, within the sediment layer. Well D15 was located approximately 0.5 km southwest of well 8801D. The subsurface atmosphere was sampled by obtaining gas from interbed and basalt zones from each of two wells in August 1988. Surface soil samples were obtained from ca. 10 cm deep in the topsoil in April 1988 (0.4 km south of 8801D) and were collected with a scoop and handled and analyzed as described above.

A 5-g (wet weight) portion of each sample was added to 45 ml of phosphate-buffered saline in a Waring blender cup and then blended for 1 min at high speed and decanted to a 125-ml flask. Slurries were fixed with 2.0 ml of 25% glutaraldehyde and allowed to settle for 24 h. Settling of particulate matter did not significantly alter the numbers of cells in the cleared liquid. After settling, 5 to 10 ml was pipetted from the cleared liquid and filtered onto a black polycarbonate filter (0.2  $\mu\text{m}$ ; Nuclepore). The filter was then stained with 0.01% acridine orange for 2 min and sandwiched

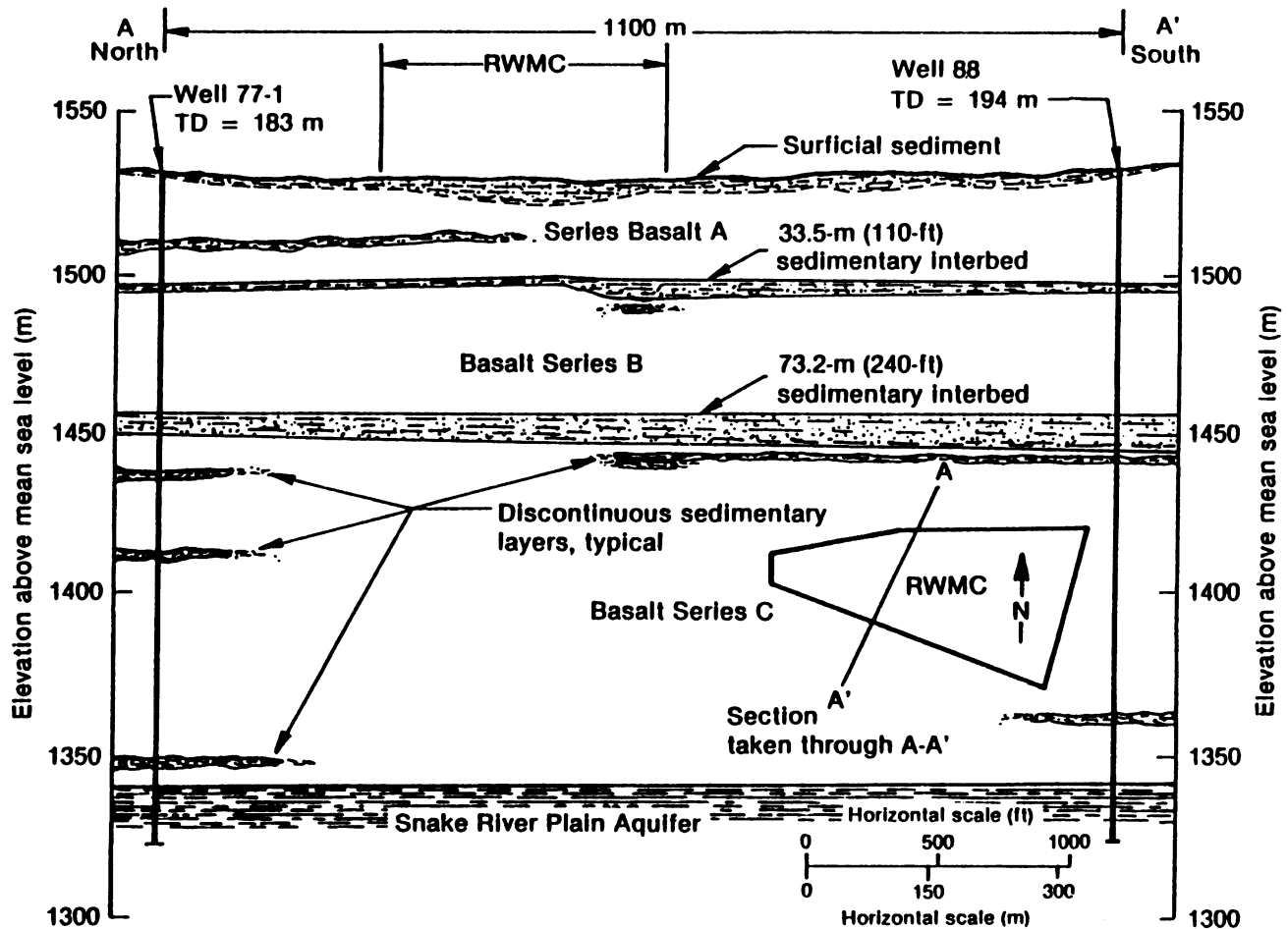


FIG. 1. Geological cross section of the Radioactive Waste Management Complex (RWMC) at the Idaho National Engineering Laboratory. Vertical exaggeration is four times the horizontal scale. Datum is mean sea level. TD, Total depth.

between a slide and a cover slip, using low-fluorescence immersion oil (Nikon). The best results were obtained when filters were not dried prior to mounting. Green fluorescing cells were counted with a Zeiss Universal microscope with epifluorescence illumination (50-W halogen lamp, 450 to 490 band-pass filter, 510 beam splitter, 520 barrier filter). Three replicate slides were prepared from each soil slurry. Controls were prepared with only phosphate-buffered saline in the same steps described above. All glassware was sterile and rinsed (twice) in prefiltered (0.2  $\mu\text{m}$ ) deionized water.

Plate counts were performed by using the spread plate technique (10) with phosphate-buffered saline as a diluent and three different microbial growth media: peptone-tryptic-yeast extract-glucose agar (PTYG) (1), a 1% (by weight) concentration of the organic constituents in PTYG (except agar), and actinomycete agar (Difco Laboratories). The initial dilution was blended as described above. Colonies were counted after 14-day incubations at  $22 \pm 2^\circ\text{C}$  and subsequently isolated onto PTYG agar. Isolates were distinguished on the basis of colony and cell morphologies, using characters described in Kolbel-Boelke et al. (12).

Soil water content and pH were estimated by conventional methods (7, 13). The organic content of surface and subsurface samples was determined with a total organic carbon analyzer (O.I. Corp.). Subsurface gas measurements (oxygen, methane, and carbon dioxide) were made with a Gow-

Mac gas chromatograph with a thermal conductivity detector and an Alltech 8700 CTRI column.

The possibility of core contamination with surface microorganisms was reduced by avoiding the use of drilling muds and by prefiltering drilling air with HEPA filters, which remove airborne particles of  $>0.3 \mu\text{m}$ . Thus, the samples obtained should reflect the actual numbers and types of microbes present in this subsurface environment.

Direct counts indicated that the surface soils studied had higher numbers of total organisms than subsurface samples:  $2.6 \times 10^6 \pm 0.4$ ,  $4.8 \times 10^5 \pm 0.3$ , and  $1.4 \times 10^5 \pm 0.3$  cells per g (dry weight)  $\pm$  standard deviation for surface, 70.1-m, and 70.4-m samples, respectively. Cell numbers estimated by plate counts showed a more marked decrease in numbers when surface soils were compared with subsurface sediments from well 8801D: on PTYG medium,  $3.5 \times 10^5 \pm 1.0$ , 50, and  $21 \pm 21$  CFU/g (dry weight) for surface, 70.1-m, and 70.4-m samples, respectively. Similar results were obtained with 1% PTYG. Bacteria were not detected by the plate count method on the sample from well D15.

The presence of microorganisms in subsurface environments deeper than topsoil habitats has been demonstrated previously, yet most studies have been conducted on samples from saturated systems in climates that receive considerable annual rainfall (1, 3, 6, 15). Because the 70-m interbed exists approximately 107 m above the aquifer, the only

apparent route for microbial colonization is through percolation of water from the surface. Such percolation likely occurred during flooding due to spring snow melt and rains as recorded in 1962, 1969, and 1982 (14) and in the pre-recorded past (2). Hypothetically, standing water on the surface enters the subsurface via fracture networks in the basalt. Visual evidence of drainage was seen as mineral deposits or caliche due to evaporation of percolation water at the basalt-sediment interface at the 70.1-m depth. Permeability of these basalts is estimated to be 1 darcy (J. C. Walton, R. G. Baca, and T. L. Rasmussen, Proceedings of Waste Management 89, Tucson, Ariz., 1989, p. 775-780), a value sufficient to transport bacteria through fractures (5).

The observed decrease in cell numbers (by direct or plate counts) with increasing depth suggests that superficial layers of basalts and sediments may filter microbes out of percolating water. Alternatively, lower bacterial numbers at greater depths may indicate locally adverse conditions (e.g., presence of toxic compounds or limited nutrients) which would reduce growth or survival of aerobic heterotrophic bacteria.

The low numbers of microbes obtained on plate counts relative to direct counts of deep sediment samples suggest that the microbes enumerated in direct counts may be either nonviable or unable to grow on the media if they have special requirements (e.g., nutritional, Eh, O<sub>2</sub> tension, or temperature). In previous studies, aquifer sediments or unsaturated sediments that are within 1 to 2 m of a saturated sediment have yielded a high percentage of cells in direct counts that are also viable (1, 3, 15). The results reported herein are similar to those reported by Ghiorse and Balkwill (8) in that plate counts were markedly lower than direct counts for unsaturated and saturated subsurface samples.

Thirty-two chemoheterotrophic bacterial isolates were obtained from media used to enumerate microbes from well 8801D. Of these, 24 were isolated from media inoculated with the basalt-sediment interface sample (70.1 m). This suggests that lower microbial diversities may exist within the interbed sediments than at the interface zone. The isolates were primarily gram positive (84%) and strict aerobes (79%). No actinomycetes were isolated from the subsurface. The predominance of gram-positive isolates may be typical of microbial populations of deep, unsaturated zones in arid environments. Other studies of subsurface environments report high numbers of pseudomonad-like isolates (15) and gram-positive isolates from sediments that represent only 43% of the total isolates from a sample (11).

The slightly alkaline pH values obtained in this study (7.9 for subsurface, 7.4 for surface soils) are typical of values in arid regions. Total organic carbon values for surface (0.26% of dry weight) and subsurface (0.05 to 0.17%) samples at this site are below values reported in the literature for various soils (see reference 17). The high water content measured in subsurface samples, 25.7% water versus 6.3% in surface soil, may allow extended microbial survival (16).

Subsurface gas concentrations were similar to air, although in one case carbon dioxide concentration was 0.16%. Methane was not detected in any of the samples (detection limit, 1 µg/ml). This suggests that the interbed zones of the subsurface are aerobic. The absence of methane is in contrast to findings of Chapelle et al. (6) that suggest deep subsurface methanogenesis in saturated coastal sediments. Bacteria with aerobic metabolism are likely to be the most active microbes in the unsaturated subsurface sediments, except in microzones of low oxygen concentration.

In summary, bacteria were found in samples from unsat-

urated, deep sediments obtained from beneath approximately 70 m of fractured basalts and sediments. Both culturable and, to a lesser extent, total numbers of bacteria decreased with depth. The presence and localized distribution of these bacteria in an arid climate may be explained if colonization of the subsurface occurred via draining of water from surface soils through fractures in the basalts. Thirty-two aerobic bacteria, predominantly gram positive and strictly aerobic, were isolated from the 70-m interbed. Gas measurements indicated that the subsurface is likely aerobic and that interbed sediments have a neutral pH, low organic carbon concentrations, and high water content. This study provides evidence of microbial survival in a subsurface habitat different from those studied previously and highly relevant with regard to waste storage sites and future attempts at bioremediation.

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